



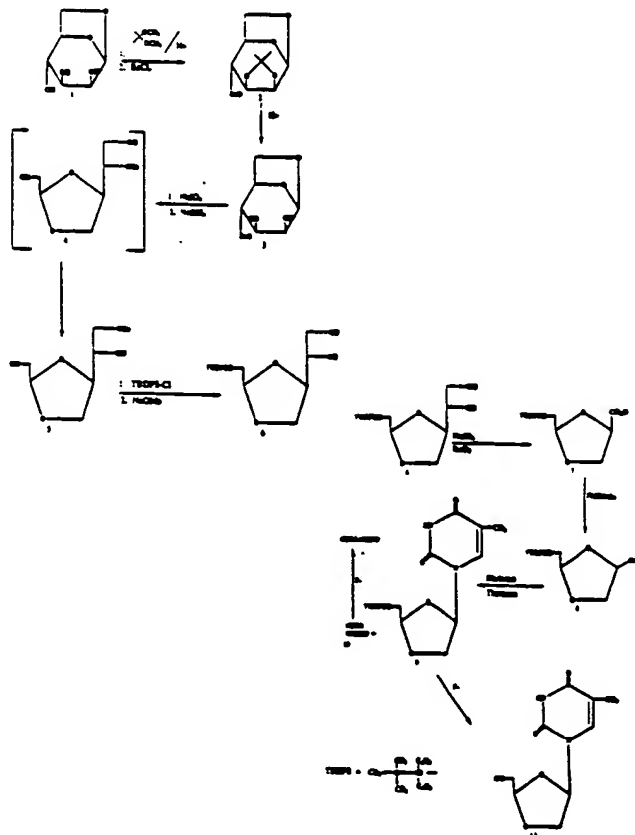
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : C07D 475/00, 487/00, 239/02 C07D 405/04, 473/00 A61K 31/52, 31/505	A1	(11) International Publication Number: WO 92/10497 (43) International Publication Date: 25 June 1992 (25.06.92)
(21) International Application Number: PCT/US91/09124 (22) International Filing Date: 5 December 1991 (05.12.91) (30) Priority data: 622,762 5 December 1990 (05.12.90) US (71) Applicants: UNIVERSITY OF GEORGIA RESEARCH FOUNDATION, INC. [US/US]; Boyd Graduate Studies Research Center, Athens, GA 30602 (US). EMORY UNIVERSITY [US/US]; 1380 S. Oxford Road, Atlanta, GA 30322 (US). (72) Inventors: CHUNG, K., Chu : 120 Orchard Knob Lane, Athens, GA 30605 (US). RAYMOND, F., Schinazi : 1524 Regency Walk Drive, Decatur, GA 30333 (US).	(74) Agents: PRATT, John, S. et al.; Kilpatrick & Cody, 100 Peachtree Street, Suite 3100, Atlanta, GA 30303 (US). (81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), MC (European patent), NL (European patent), SE (European patent). Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	

(54) Title: ENANTIOMERICALLY PURE β -D-(-)-DIOXOLANE-NUCLEOSIDES

(57) Abstract

An asymmetric process for the preparation of enantiomerically pure β -D-(-)-dioxolane-nucleosides. The enantiomerically pure dioxolane nucleosides are active HIV agents, that are significantly more effective than the prior prepared racemic mixtures of the nucleosides. The anti-viral activity of the compounds is surprising in light of the generally accepted theory that moieties in the endo conformation, including these dioxolanes, are not effective antiviral agents. The toxicity of the enantiomerically pure dioxolane nucleosides is lower than that of the racemic mixture of the nucleosides, because the nonnaturally occurring α -isomer is not included. The product can be used as a research tool to study the inhibition of HIV *in vitro* or can be administered in a pharmaceutical composition to inhibit the growth of HIV *in vivo*.



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ENANTIOMERICALLY PURE β -D-(-)-DIOXOLANE-NUCLEOSIDES

The government has rights in this invention by virtue of grants from the Public Health Service of the National Institute of Allergy and Infectious Diseases, and the Department of Veteran's Affairs that partially funded research leading to this invention.

Background of the Invention

This invention is in the area of organic synthesis of nucleosides, and in particular relates to a process for the preparation of enantiomerically pure β -D-(-)-dioxolane nucleosides.

A number of 2',3'-dideoxynucleosides have been found to be potent antiviral agents against human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS). The lead compound, AZT (Mitsuya, H.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.*, 1986 83, 1911) has been approved by the U.S. Food and Drug Administration for patients with AIDS and AIDS-related complex. Several other 2',3'-dideoxynucleosides are undergoing various stages of clinical trials, including 3'-azido-2',3'-dideoxyuridine (AZDU or CS-87, see, Chu, C.K.; et al., *J. Med. Chem.*, 1989, 32, 612; and Eriksson, B.F.H.; et al., *Antimicrob. Agents Chemother.*, 1989, 33, 1927), 2',3'-dideoxyinosine (DDI) and 2',3'-dideoxycytidine (DDC) (see Yarchoan, R. et. al., *Science*, 1989, 245, 412), 3'-deoxy-2',3'-didehydrothymidine (D4T, Lin, T.S., et al., *Biochem. Pharmacol.*, 1987, 36, 311; Hamamoto, Y., et al., *Antimicrob. Agents Chemother.*, 1987, 31, 907; Balzarini, J., et al., *Biochem. Biophys. Res. Commun.*, 1987, 140, 735), and 2'-fluoro-arabinofuranosyl-2'-3'-dideoxycytidine (Martin, T.A., et al., *J. Med. Chem.*, 1990, 33, 2137; Watanabe, K.A.,

et al., *J. Med. Chem.*, 1990, 33, 2145; Sterzycki, R.Z., et al., *J. Med. Chem.*, 1990 33, 2150).

In the 5'-triphosphorylated form, these nucleosides are known to inhibit HIV reverse transcriptase as well as cause chain-termination of the growing viral DNA chain. Furman, P.A., et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1986, 83, 8333; Cheng, Y.C., et al., *J. Biol. Chem.*, 1987, 262, 2187; St. Clair, M.H., et al., *Antimicrob. Agents Chemother.*, 1987, 31, 1972; and Schinazi, R.F., et al., *Antimicrob. Agents Chemother.*, 1989 33, 115.

The stereochemistry of nucleoside derivatives play an important role in their biological activity. The C1' position of the ribose in the nucleoside (the carbon bound to the nitrogen of the heterocyclic base) is a chiral center because the carbon is attached to four different moieties. Likewise, there is an optically active center at C4' of the nucleoside (the ring carbon bound to the hydroxymethyl group that is phosphorylated in nucleotides). In the naturally occurring nucleosides, both the base attached to the C1' atom and the hydroxymethyl group attached to the C4' atom are on the same side of the carbohydrate ring.

A carbohydrate configuration in which the C1' and C4'-substituents are on the same side of the carbohydrate plane (i.e., the substituents are cis) is referred to as a " β -configuration." A carbohydrate configuration in which the C1' and C4'-substituents are on the opposite side of the carbohydrate plane (i.e., the substituents are trans) is referred to as an " α -configuration". Referring to compound 1 of Figure 2, a nucleoside is designated a D-nucleoside if the non-hydrogen substituent attached to the C4'-atom is above the plane of the carbohydrate ring. The nucleoside is designated an L-nucleoside if the non-hydrogen

substituent attached to the C4'-atom is below the plane of the carbohydrate ring.

5 The non-naturally occurring α -isomers of nucleosides (in which the C1' or C4' substituents are on opposite sides of the carbohydrate plane) are rarely biologically active, and are typically toxic.

10 An analysis of the solid-state conformations of six active and two inactive anti-HIV nucleoside agents was recently performed to attempt to correlate the presence or absence of certain stereochemical features with high HIV activity. Van Roey, P., et al., *J. Am. Chem. Soc.*, 1988, 110, 2277; and Van Roey, P., et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1989, 86, 3929. The x-ray structures indicated that active anti-HIV nucleosides assume the
15 C3'-exo or similar carbohydrate conformations while inactive compounds prefer the C3'-endo conformation. (Endo and exo refer to the conformations in which the atoms are at the same or opposite side of the sugar ring in relation to the base). The C3'-exo and C3'-endo
20 conformations place the C5' atom in axial and equatorial positions, respectively. The position of the C5' atom affects the location of the 5'-hydroxyl group in relation to the base. Since the 5'-hydroxyl group is the site of phosphorylation of the nucleoside, its
25 location with respect to the rest of the nucleoside is important.

There has been recent interest in the synthesis of nucleoside derivatives in which the 3'-carbon of the nucleoside has been replaced with a heteroatom.
30 Norbeck, D.W., et al., in *Tet. Lett.*, 1989, 30, 6263, reported the synthesis of (\pm)-1-[(2B,4B)-2-(hydroxymethyl)-4-dioxolanyl]thymine (referred to below as (\pm)-dioxolane-T, see Figure 1), that results in a racemic mixture of diastereomers about the C4' atom.
35 The product is a derivative of 3'-deoxythymidine in

which the C3' atom has been replaced with an O3' atom. The product was synthesized in five steps from benzyloxyaldehyde dimethylacetal and (\pm)-methyl glycerate to produce a 79% yield of the 1:1
5 diastereomeric mixture. The X-ray crystallographic analysis of the product revealed that the dioxolane ring adopts the 3T_4 conformation commonly observed in ribonucleosides, with the O3' atom in the endo position. Norbeck reported that the racemic mixture of dioxolane-
10 T exhibits an anti-HIV activity of 20 μ M in ATH8 cells, and attributed the low efficacy against the virus to an effect of the endo conformation of the O3' atom.

Belleau, et al., in the Fifth International Conf. on AIDS, Montreal, Canada June 4-9, 1990, paper No.
15 T.C.O.1., reported a method of synthesis of cytidine nucleosides that contain oxygen or sulfur in the 3'-position. The dioxolane ring was prepared by the condensation of $\text{RCO}_2\text{CH}_2\text{CHO}$ with glycerine. As with the Norbeck synthesis, the Belleau synthesis results in a
20 racemic mixture of diastereoisomers about the C4' carbon of the nucleoside. Belleau reported that the sulfur analog, referred to as NGBP-21 or (\pm) BCH-189 (see Figure 1), had high anti-HIV activity. (\pm) BCH-189 is currently undergoing preclinical toxicology.

25 To date, no one has reported a method of synthesis of a nucleoside analog with an oxygen in the 3'-position that results in an enantiomerically pure dioxolane nucleoside that has the same stereochemistry as the nucleosides found in nature (the β stereoisomer). There
30 is a need for such a synthesis as a research tool to provide more information on the effect of stereochemistry on the anti-viral activity of nucleoside derivatives, and to provide new anti-HIV agents.

It is therefore an object of the present invention
35 to provide a method of synthesis of enantiomerically

pure dioxolane nucleosides.

It is another object of the present invention to provide enantiomerically pure dioxolane nucleosides with significant anti-HIV activity.

5

Summary of the Invention

The invention as disclosed is an asymmetric process for the preparation of enantiomerically pure β -D-(-)-dioxolane-nucleosides. The process involves the initial preparation of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane from 1,6-anhydromannose, a sugar that contains all of the necessary stereochemistry for the enantiomerically pure final product, including the correct diastereomeric configuration about the 1 position of the sugar (that becomes the 4'-position in the later formed nucleoside).

The (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane is condensed with a desired heterocyclic base in the presence of SnCl_4 , other Lewis acid, or trimethylsilyl triflate in an organic solvent such as dichloroethane, acetonitrile, or methylene chloride, to provide the stereochemically pure dioxolane-nucleoside.

The enantiomerically pure dioxolane nucleosides are active HIV agents, that are significantly more effective than the prior prepared racemic mixtures of the compounds. The anti-viral activity of the compounds is surprising in light of the generally accepted theory that moieties in the endo conformation, including these dioxolanes, are not effective antiviral agents. Further, the enantiomerically pure dioxolane nucleosides are less toxic than the racemic mixture of nucleosides because the nonnaturally occurring isomer has been eliminated.

The product can be used as a research tool to study the inhibition of HIV in vitro or can be administered in a pharmaceutical composition to inhibit the growth of HIV in vivo.

5

Brief Description of the Figures

Figure 1 is an illustration of the chemical structures of (\pm) -1-[(2 β ,4 β)-2-(hydroxymethyl)-4-dioxolanyl]thymine (dioxolane-T) and (\pm) -1-[(2 β ,4 β)-2-(hydroxymethyl)-4-(1,3-thioxolane)]thymine (BCH-189).

10

Figure 2 is an illustration of the method of synthesis of enantiomerically pure β -D-(-)-dioxolane-thymine.

15

Detailed Description of the Invention

As used herein, the term "protected" refers to a moiety that has been placed on a functional group of a molecule to prevent further reaction of the moiety during derivatization of another portion of the molecule. Protecting groups, particularly for oxygen and nitrogen, are well known to those skilled in the art of organic chemistry.

20

The term "1,3-dioxolane nucleoside" as used herein refers to a nucleoside derivative as depicted in Figures 1 and 2, wherein a 1,3-dioxolane is attached to a heterocyclic base, typically a purine or pyrimidine base, through the oxathiolane C5 carbon (that becomes the C1'-carbon in the nucleoside).

25

I. Preparation of Enantiomerically Pure Dioxolane Nucleosides

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In preparing enantiomerically pure dioxolane nucleosides, care should be taken to avoid strong acidic

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conditions that would cleave the dioxolane ring. Reactions should be performed, if possible, in basic or neutral conditions, and when acidic conditions are necessary, the time of reaction should be minimized.

5

A. Preparation of Dioxolane Derivative

The key starting material for the synthesis of enantiomerically pure β -D-(-)-dioxolane-nucleosides is
10 1,6-anhydromannose (compound 1, Figure 2). This sugar contains all of the necessary stereochemistry for the enantiomerically pure final product (see for example, compound 11, Figure 2), including the correct diastereomeric configuration about the 1 position of the
15 sugar (that becomes the 4'-position in the later formed nucleoside). 1,6-Anhydromannose can be prepared according to procedures described in Knauf, A.E.; Hann, R.M.; Hudson, C.S. *J. Am. Chem. Soc.*, 1941, 63, 1447; and Zottola, M.A.; Alonso, R.; Vite, G.D.; Fraser-Reid,
20 B. *J. Org. Chem.*, 1989, 54, 6123. Prior syntheses of dioxolane nucleosides have used racemic mixtures of starting materials for the preparation of the ribose moiety. When the syntheses begin with a racemic mixture of reagents, undesirable racemic mixtures of
25 enantiomeric nucleoside products have been produced. The mixtures are very difficult to separate and significantly increase the cost of the final product. Further, the inclusion of nonnaturally occurring isomers increases the toxicity of the product.

30 The 1,6-anhydromannose is converted to its isopropylidene derivative with dimethoxypropane and p-toluenesulfonic acid, which, without isolation, is benzoylated in the 4-position to compound 2 (see Figure 2). An acyl group can also be used to protect the 4-
35 position. The isopropylidene group of compound 2 is

when the condensation reaction is carried out with a 1,3-thioxolane.

Functional oxygen and nitrogen groups on the heterocyclic base should be protected before
5 condensation with the sugar. Protecting groups are well known to those skilled in the art, and include trimethylsilyl, dimethylhexylsilyl, t-butyl-
dimethylsilyl, and t-butyl-diphenylsilyl, tritylmethyl, alkyl groups, acyl groups (lower alkyl-
10 C(O)) such as acetyl and propionyl, methylsulfonyl, and p-toluylsulfonyl.

Friedel-Crafts catalysts (Lewis acids) that can be used in the condensation reaction include SnCl_4 , ZnCl_4 , TiCl_4 , AlCl_3 , FeCl_3 , BF_3 -diethylether, and BCl_3 . These
15 catalysts require anhydrous conditions because the presence of water reduces their activity. The catalysts are also inactivated in the presence of organic solvents with active hydrogens, such as alcohols and organic acids. The catalysts are typically used in solvents
20 such as carbon disulfide, methylene chloride, nitromethane, 1,2-dichloroethane, nitrobenzene, tetrachloroethane, chlorobenzene, benzene, toluene, dimethylformamide, tetrahydrofuran, dioxane, or acetonitrile. Anhydrous aluminum chloride is not
25 soluble in carbon disulfide. Niedballa, et al., J. Org. Chem. 39, 25 (1974). The preferred catalyst is SnCl_4 . The preferred solvent is 1,2-dichloroethane.

Trimethylsilyl triflate can be used under the same conditions described above for the Friedel-Crafts
30 catalysts. The reaction proceeds at a temperature range of from -10°C to 200°C .

The choice of catalyst for condensation will affect the final product ratio of α to β nucleoside product. For example, condensation of the intermediates (2R,4R)-
35 and (2R,4S)-4-acetoxy-2-(t-butyl-diphenylsilyloxymethyl)

dioxolane (compound 8, Figure 2) with silylated thymidine in the presence of trimethylsilyl triflate in CH_2Cl_2 gave a mixture of (-)-1-[(2R,4R)-2-(t-butyl-diphenylsilyloxymethyl)-4-dioxolanyl]thymine 9- β (45%) and (+)-1-[(2R,4S)-2-(t-butyl-diphenylsilyloxymethyl)-4-dioxolanyl] thymine 10- α (29%). However, the reaction with SnCl_4 produced exclusively β -isomer 9 with trace amounts of α -isomer 10 detectable on TLC.

In the final step of this method of preparation of enantiomerically pure (-)- β -D-dioxolane-nucleosides, the 5'-O-position of the nucleoside is deprotected. Desilylation can be carried out with a variety of reagents, including acetic acid, trifluoroacetic acid, hydrogen fluoride, n-tetrabutylammonium fluoride, potassium fluoride and pyridinium HCl. For example, desilylation of compounds 9 and 10 with tetrabutylammonium fluoride gave the desired free nucleosides 11 and 12, respectively (Figure 2). Acetic acid is preferred for commercial scale use because it is inexpensive. Other reagents for desilylation are known to those skilled in the art. Deacylation is accomplished in acid or base. 5-O-Ethers can be cleaved with BCl_3 or trimethylsilyl iodide.

The method of preparation of enantiomerically pure (-)- β -D-dioxolane-nucleosides is further illustrated in the following working example for the preparation of (-)-1-[(2S,4S)-2-(hydroxymethyl)-4-dioxolanyl]thymine, referred to as (-)- β -D-dioxolane-T. The enumeration of compounds in the working examples refer to structures set out in Figure 2.

(-)-1,6-Anhydro-2,3-isopropylidene-4-O-benzoyl- β -D-mannopyranose

1,6-anhydro- β -D-mannopyranose (compound 1) was mixed
5 with acetone (800 ml) and methanol (300 ml) and stirred
for approximately thirty minutes until only a free-
flowing solid remained. Dimethoxypropane (300 ml), and
p-toluenesulfonic acid (5 g) were then added, and the
mixture stirred for 2 hours.

10 The reaction mixture was then made basic with
triethylamine (pH 8), and filtered to remove the white
solid material. The solvents were evaporated, and the
residue taken up in ethyl acetate and then crystallized
to obtain 4 grams of the 2,3-isopropylidenated product
15 as clear needles.

To a solution of the 1,6-anhydro-2,3-isopropylidene-
 β -D-mannopyranose (5.01 g, 0.025 mol) in pyridine (40
ml) was added dropwise benzoyl chloride (3.74 ml, 0.062
mol) at 0°C. The mixture was stirred for 45 minutes at
20 0°C. Ice was then added to the reaction mixture to
remove excess of benzoyl chloride. The solvent was
evaporated under vacuum and the residue was dissolved
in ethyl acetate (200 ml). The organic layer was washed
with water, sat. NaHCO₃ and brine. The resulting
25 material was dried over anhydrous MgSO₄, filtered, and
then evaporated to give (-)-1,6-anhydro-2,3-
isopropylidene-4-O-benzoyl- β -D-mannopyranose crude
product (compound 2, 8.7 g) as yellowish solid.

30 **(-)-1,6-Anhydro-4-O-benzoyl- β -D-mannopyranose (3).**

To a solution of 1,6-anhydro-4-O-benzoyl-2,3-
isopropylidene- β -D-mannopyranose 2 (10.0 g, 32.6 mmole)
in 60% aqueous dioxane (820 ml) was added concentrated
H₂SO₄ (3.36 ml). The mixture was stirred at 70-80° C
35 for 15 hours, and then cooled in an ice bath,
neutralized with NaHCO₃ and concentrated until half of

the original volume remained. The solution was then extracted with ethyl acetate and the combined organic layers washed with saturated NaHCO_3 solution and water, dried, and evaporated to give 3 as a white solid. The solid was crystallized from CH_2Cl_2 -n-hexane to yield 3 (7.4 g, 85.3%) as white solid: $[\alpha]^{25}_{\text{D}} - 154.7^\circ$ (C, 0.21 MeOH); ^1H NMR ($\text{DMSO}-d_6$): δ 3.56-4.61 (m, 5H, 2,3,5,6-H), 4.82 (d, $J=8.1$ Hz, 1H, OH D_2O exchangeable), 5.02 (s, 1H, 4-H), 5.09 (d, $J=3.7$ Hz, 1H, OH, D_2O exchangeable), 5.28 (s, 1H, 1-H), 7.46-8.05 (m, 5H, Ar-H); IR (KBr) 3410, 1710 cm^{-1} ; Anal. Calcd for $\text{C}_{13}\text{H}_{14}\text{O}_6$: C, 58.64; H, 5.31. Found: C, 58.51; H, 5.34.

(-)-(2R,4R)-4-(2-Benzoxymethyl)-2-(hydroxymethyl)dioxolane (5).

To a solution of 3 (7.4 g, 27.8 mmole) in 95% ethanol (200 ml) was added a solution of NaIO_4 (6.54 g, 30.7 mmole) in water (200 ml). The mixture was stirred at room temperature for 1 hour. After checking to insure the complete conversion of diol to dialdehyde by thin layer chromatography, the reaction mixture was concentrated to the half of the original volume. Methanol (200 ml) was added to the residue and the mixture was cooled to 50°C . Sodium borohydride (4.2 g, 111.0 mmole) was added to the mixture portion-wise for 5 minutes and the mixture was stirred at 50°C for 10 minutes, neutralized with glacial acetic acid and concentrated to yield crude 3 as yellow oil. The oil was purified by column chromatography over silica gel to yield pure 3 as colorless oil, that was crystallized from diethyl ether/n-hexane to yield 5 (6.12 g, 82%) as white solid: $[\alpha]^{25}_{\text{D}} - 18.5^\circ$ (C 0.20, methanol); ^1H NMR ($\text{DMSO}-d_6$): δ 3.47 (dd, $J=5.9, 3.7$ Hz, 2H, CH_2OH), 3.72-4.14 (m, 4H, 4, 5-H and CHOH), 4.27-4.95 (m, 2H, CH_2OBz), 4.81-4.95 (m, 2-H and pri OH), 5.43 (d, $J=5.5$

Hz, 1H, sec OH, D₂O exchangeable), 7.43-8.09 (m, 5H, Ar-H); Anal. Calcd for C₁₃H₁₆O₆: C, 58.19; H, 6.02. Found: C, 58.09; H, 6.01.

5 **(-)-(2R,4R)-4-(2-Benzoxo-1-hydroxyethyl)-2-(t-butyl-diphenylsilyloxy-methyl)-dioxolane.**

To a solution of **3** (2.8 g, 10.4 mmole) and imidazole (2.04 g, 30.0 mmole) in dimethylformamide (40 ml) was
10 added t-butyl-diphenylsilyl chloride (3 ml, 11.5 mmole). The mixture was stirred at room temperature for 2 hours. The reaction mixture was evaporated to yield a yellow oil, that was purified by column chromatography over silica gel to yield **4** (4.48 g, 85%) as a colorless oil;
15 [α]²⁵_D - 14.2° (C 0.26, methanol); ¹H NMR (DMSO-d₆): δ 1.00 (s, 9H, t-Bu), 3.68-3.87 (m, 3H, CH₂OTBDPS and CHOH), 3.98-4.16 (m, 3H, 4,5-H), 4.20-4.55 (m, 2H, CH₂OBz), 5.07 (t, J=3.3 Hz, 1H, 2-H), 5.47 (d, J=5.7 Hz, 1H, OH, D₂O exchangeable), 7.40-8.33 (m, 10H, Ar-H);
20 Anal. Calcd for C₂₉H₃₄O₆Si: C, 68.73; H, 6.79. Found: C, 68.86; H, 6.83.

25 **(-)-(2R,4R)-2-(t-Butyl-diphenylsilyloxymethyl)-4-(1,2-dihydroxyethyl)-dioxolane (6).**

To a solution of (-)-(2R,4R)-4-(2-benzoxo-1-hydroxyethyl)-2-(t-butyl-diphenylsilyloxy-methyl)-dioxolane (2.52 g, 5.0 mmole) in methanol (40 ml) was
30 added a 0.078 M solution of sodium methoxide (7.3 ml) in methanol. The mixture stirred at room temperature for two hours. The mixture was neutralized with acetic acid and concentrated. The residue was then portioned between ethyl acetate and water, and the aqueous layer extracted with ethyl acetate. The combined organic
35 layers were washed with a saturated NaHCO₃ solution and then water, and then dried, evaporated, and purified by column chromatography over silica gel to yield **6** (1.9

g, 95%) as colorless oil: $[\alpha]^{25}_D - 2^\circ$ (C 0.25, MeOH); ^1H NMR (DMSO- d_6) δ 1.00 (s, 9H, t-Bu), 3.40-3.52 (m, 3H, CH_2OH and CHOH), 3.64 (d, $J=3.7$ Hz, 2H, CH_2OTBDPS), 3.82-3.95 (m, 3H, 4.5-H), 4.49 (t, $J=5.3$ Hz, 1H, pri OH, D_2O exchangeable), 4.82 (d, $J=5.1$ Hz, 1H, sec OH, D_2O exchangeable), 5.01 (t, $J=3.7$ Hz, 1H, 2-H), 7.36-7.71 (m, 10H, Ar-H); Anal. Calcd for $\text{C}_{22}\text{H}_{33}\text{H}_{30}\text{O}_5\text{Si}$: C, 65.63; H, 7.53. Found: C, 65.72; H, 7.52.

10 **(+)-(2R,4R)-2-(t-Butyldiphenylsilyloxymethyl)-4-carboxyldioxolane (7).**

To a biphasic solution of **6** (1.6 g, 4.0 mmole) in CH_3CN (8 ml), CCl_4 (8 ml) and H_2O (12 ml) was added NaIO_4 (3.59 g, 16.8 mmole) and RuO_2 hydrate (8.5 mg). The mixture was vigorously stirred at room temperature for 5 hours. Methylene chloride (40 ml) was added to the mixture. The organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 . The combined organic layers were washed with water, filtered through celite pad and then concentrated to yield crude **7** (1.2 g, 77.4%) as black oil, that was used in the next reaction without further purification. For analytical purposes crude **7** was purified by column chromatography over silica gel to yield **7** as a white foam: $[\alpha]^{25}_D + 15.7^\circ$ (C 0.28, MeOH); ^1H NMR (DMSO- d_6) δ 0.99 (s, 9H, t-Bu), 3.43-4.05 (m, 4H, 5-H and CH_2OTBDPS), 4.25 (t, $J=6.8$ Hz, 1H, 4-H), 5.04 (dd, $J=5.1, 3.7$ Hz, 1H, 2-H), 7.38-7.72 (m, 10H, Ar-H).

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(2R,4R)- and (2R,4S)-4-Acetoxy-2-(t-butyldiphenylsilyloxymethyl) dioxolane (8).

To a solution of **7** (0.46 g, 1.14 mmole) in ethyl acetate (10 ml) was added pyridine (0.09 ml, 1.25 mmole) and $\text{Pb}(\text{OAc})_4$ (0.66 g, 1.49 mmole). The mixture was stirred at room temperature for 15 hours under N_2 , and

35

then filtered through celite pad, and then concentrated and purified by column chromatography over silica gel to yield 8 (0.29 g, 63.5%) as a colorless oil: ¹H NMR (CDCl₃) δ 1.06 and 1.10 (s, 9H, t-Bu), 1.92 and 2.06 (s, 1H, CH₃), 3.71-4.24 (m, 4H, 5-H and CH₂OTBDPS), 5.25 and 5.38 (t, J=4.3 and 3.3 Hz each, 1H, 2-H), 6.27-6.41 (m, 1H, 4-H), 7.20-7.72 (m, 10H, Ar-H); IR (KBr) 3400, 1620 cm⁻¹.

10 (-)-1-[(2R,4R)-2-(t-Butyldiphenylsilyloxymethyl)-4-dioxolanyl]thymine (9) and (+)-1-[(2R,4S)-2-(t-Butyldiphenylsilyloxymethyl)-4-dioxolanyl] thymine (10).

To a suspension of thymine (0.15 g, 1.2 mmole) in
15 hexamethyldisilazane (10 ml) was added a catalytic amount of (NH₄)₂SO₄, and the mixture refluxed for 3 hours. The clear solution obtained was concentrated to yield silylated thymine as a colorless oil. A solution of 8 (0.24 g, 0.6 mmole) in CH₂Cl₂ (5 ml) was added to
20 a solution of silylated thymine in CH₂Cl₂ (5 ml) and the mixture cooled to 5°C. To the cooled mixture was added trimethylsilyl triflate (0.23 ml, 1.2 mmole), and the mixture stirred at room temperature for 1 hour under N₂. A saturated NaHCO₃ solution (20 ml) was added to the
25 mixture, and the mixture again stirred at room temperature for 30 minutes. The organic layer was then separated and the aqueous layer extracted with CH₂Cl₂. The combined organic layer was washed with a saturated NaHCO₃ solution and water, dried, concentrated and
30 separated by column chromatography over silical gel to yield 9 (0.125 g, 44.6%) as white foam and 10 (0.08 g, 28.6%) as white foam: 9 (β-form); [α]²⁵D - 6.98° (C 0.43, MeOH); ¹H NMR (CDCl₃) δ 1.08 (s, 9H, t-Bu), 1.67 (s, 3H, CH₃), 3.92 (d, J=3.2 Hz, 2H, CH₂OTBDPS), 4.14 (d, J=4.0
35 Hz, 2H, 5-H), 5.06 (t, J=3.2 Hz, 1H, 2-H), 6.36 (t, J=4.0 Hz, 1H, 4-H), 7.26-7.75 (m, 10H, Ar-H), 9.51 (bnr

s, 1H, H=NH): UV (MeOH) λ_{\max} 265.0 (pH 2); 264.4 nm (pH 11); Anal. Calcd for $C_{25}H_{30}O_5N_2Si$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.28; H, 6.51; N, 5.98:

5 10 (α -form); $[\alpha]^{25}_D + 11.3^\circ$ (C 0.23, MeOH); 1H NMR (CDCl₃) δ 1.08 (s, 9H, t-Bu), 1.94 (d, J=1.2 Hz, 3H, CH₃), 3.70 (d, J=3.2 Hz, 2H, CH₂OTBDPS), 4.01 (dd, J=9.5, 2.3 Hz, 1H, 5H), 4.35 (dd, J=9.5, 5.3 Hz, 1H, 5-H), 5.55 (t, J=3.2 Hz, 1H, 2-H), 6.32 (dd, J=5.3, 2.3 Hz, 1H, 4-H), 7.17 (d, J=1.2 Hz, 1H, 6'-H), 7.37-7.74 (m, 10H, Ar-H), 9.57 (br s, 1H, NH); UV (MeOH) λ_{\max} 265.0; (pH 2);
 10 264.5 nm (pH 11); Anal. Calcd for $C_{25}H_{30}O_5N_2Si$: C, 64.34; H, 6.49; N, 6.00. Found C, 64.23; H, 6.51; N, 5.93.

15 (-)-1-[(2R,4R)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (11).

To a solution of 9 (93.3 mg, 0.2 mmole) in tetrahydrofuran (THF) (3 ml) was added a 1.0 M solution of tetra-n-butylammonium fluoride in THF (0.24 ml, 0.24
 20 mmole) and the mixture stirred at room temperature for 1 hour. The mixture was then concentrated and purified by column chromatography over silica gel to yield 11 (42 mg, 92.1%) as white solid: $[\alpha]^{25}_D - 18.8^\circ$ (C 0.17, MeOH); 1H NMR (DMSO-d₆) δ 1.75 (d, J=1.2 Hz, 3H, CH₃), 3.63 (dd, J+6.0, 2.6 Hz, 2H, CH₂OH), 4.03 (dd, J=9.9, 5.5 Hz, 1H, 5-H), 4.22 (dd, J=9.9, 2.0 Hz, 1H, 5-H), 4.90 (t, J=2.6 Hz, 1H, 2-H), 5.16 (t, J-t.0 Hz, 1H, OH), 6.21 (dd, J=5.5, 2.0 Hz, 1H, 4-H), 7.67 (d, J=1.2 Hz, 1H, 6'-H), 11.27 (br s, 1H NH); UV (H₂) max 266.0 (ϵ 10757); 266.5
 25 (ϵ 9894) (pH 2); 266.3 (ϵ 8397) (pH 11); Anal. Calcd for $C_9H_{12}O_5N_2$: C, 47.36; H, 5.31; N, 12.28. found: c, 47.28; H, 5.34; N, 12.29.

(+)-1-[(2R,4S)-2-(Hydroxymethyl)-4-dioxolanyl]thymine (12).

Deprotection of 10 (60 mg, 0.13 mmole) according to same procedure as described above for 11 yielded 12 (26 mg, 87.6%) as a white foam: $[\alpha]^{25}_D + 10.7^\circ$ (C 0.15, MeOH); 1H NMR (DMSO- d_6) δ 1.79 (s, 3H, CH₃), 3.43 (dd, J=6.0, 3.7 Hz, 2H, CH₂OH), 4.02 (dd, J=9.5, 3.3 Hz, 1H, 5-H), 4.28 (dd, J=9.5, 5.6 Hz, 1H, 5-H), 5.00 (t, J=6.0 Hz, 1H, OH), 5.47 (t, J=3.7 Hz, 1H, 2-H), 6.17 (dd, J=5.6, 3.3 Hz, 1H, 4-H), 7.43 (d, J=1.2 Hz, 1H, 6'-H), 11.32 (br s, 1H NH); UV (H₂O) λ_{max} 266.5 (ϵ 9454); 266.5 (ϵ 9199) (pH 2); 266.3 (ϵ 6925) (pH=11); Anal. Calcd for C₉H₁₂O₅N₂; C, 47.36; H, 5.31; N, 12.28. found: C, 47.22; H, 5.32; N, 12.16.

II. Anti-HIV Activity of Dioxolane Nucleosides

In contrast to the previous report that β -D-(+)-dioxolane-thymine has low efficacy against HIV in ATH8 cells, the enantiomerically pure β form 11 exhibited a potent anti-HIV activity ($EC_{50} = 0.3 \mu M$). It was surprising to discover that enantiomerically pure β -D-(-)-dioxolane-T has significantly higher anti-HIV activity than the racemic mixture of the compound. This difference might be explained based on the rate of phosphorylation of 11 in these systems. As expected, the α -isomer 12 did not exhibit any significant anti-HIV activity.

β -D-(-)-Dioxolane-nucleosides can be used as research tools to inhibit the growth of HIV in vitro, or can be administered pharmaceutically to inhibit the growth of HIV in vivo.

The ability of β -D-(-)-dioxolane-nucleosides to inhibit HIV can be measured by various experimental techniques. The technique used herein, and described

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agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent
5 such as peppermint, methyl salicylate, or orange flavoring.

When the dosage unit form is a capsule, it can contain, in addition to material of the above type, a liquid carrier such as a fatty oil. In addition, dosage
10 unit forms can contain various other materials which modify the physical form of the dosage unit, for example, coatings of sugar, shellac, or other enteric agents.

β -D-(-)-Dioxolane-nucleosides or their salts can
15 be administered as a component of an elixir, suspension, syrup, wafer, chewing gum or the like. A syrup may contain, in addition to the active compounds, sucrose as a sweetening agent and certain preservatives, dyes and colorings and flavors.

β -D-(-)-Dioxolane-nucleosides or their salts can
20 also be mixed with other active materials that do not impair the desired action, or with materials that supplement the desired action, such as antibiotics, antifungals, antiinflammatories, or other antivirals,
25 including other nucleoside anti-HIV compounds.

Solutions or suspensions used for parenteral, intradermal, subcutaneous, or topical application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils,
30 polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as
35 acetates, citrates or phosphates and agents for the

adjustment of tonicity such as sodium chloride or dextrose. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

5 If administered intravenously, preferred carriers are physiological saline or phosphate buffered saline (PBS).

10 In a preferred embodiment, the active compounds are prepared with carriers that will protect the compound against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods
15 for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including
20 liposomes targeted to infected cells with monoclonal antibodies to viral antigens) are also preferred as pharmaceutically acceptable carriers. These may be prepared according to methods known to those skilled in the art, for example, as described in U.S. Patent No.
25 4,522,811 (which is incorporated herein by reference in its entirety). For example, liposome formulations may be prepared by dissolving appropriate lipid(s) (such as stearoyl phosphatidyl ethanolamine, stearoyl phosphatidyl choline, arachadoyl phosphatidyl choline, and cholesterol) in an inorganic solvent that is then
30 evaporated, leaving behind a thin film of dried lipid on the surface of the container. An aqueous solution of the active compound or its monophosphate, diphosphate, and/or triphosphate derivatives are then
35 introduced into the container. The container is then

swirled by hand to free lipid material from the sides of the container and to disperse lipid aggregates, thereby forming the liposomal suspension.

5 **V. Preparation of Phosphate Derivatives of β -D-(-)-Dioxolane-Nucleosides**

10 Mono, di, and triphosphate derivative of β -D-(-)-dioxolane-nucleosides can be prepared as described below.

15 The monophosphate can be prepared according to the procedure of Imai et al., J. Org. Chem., 34(6), 1547-1550 (June 1969). For example, about 100 mg of β -D-(-)-dioxolane-nucleoside and about 280 μ l of phosphoryl chloride are reacted with stirring in about 8 ml of dry ethyl acetate at about 0°C for about four hours. The reaction is quenched with ice. The aqueous phase is purified on an activated charcoal column, eluting with
20 5% ammonium hydroxide in a 1:1 mixture of ethanol and water. Evaporation of the eluant gives ammonium-(β -D-(-)-dioxolane-nucleoside)-5'-monophosphate.

25 The diphosphate can be prepared according to the procedure of Davisson et al., J. Org. Chem., 52(9), 1794-1801 (1987). β -D-(-)-Dioxolane-nucleosides can be prepared from the corresponding tosylate, that can be prepared, for example, by reacting the nucleoside with tosyl chloride in pyridine at room temperature for about 24 hours, working up the product in the usual manner
30 (e.g., by washing, drying, and crystallizing it).

35 The triphosphate can be prepared according to the procedure of Hoard et al., J. Am. Chem. Soc., 87(8), 1785-1788 (1965). For example, β -D-(-)-dioxolane-nucleoside is activated (by making a imidazolid, according to methods known to those skilled in the art) and treating with tributyl ammonium pyrophosphate in

DMF. The reaction gives primarily the triphosphate of the nucleoside, with some unreacted monophosphate and some diphosphate. Purification by anion exchange chromatography of a DEAE column is followed by isolation
5 of the triphosphate, e.g., as the tetrasodium salt.

This invention has been described with reference to its preferred embodiments. Variations and modifications of the invention, enantiomerically pure β -D-(-)-dioxolane-nucleosides, will be obvious to those skilled
10 in the art from the foregoing detailed description of the invention. It is intended that all of these variations and modifications be included within the scope of the appended claims.

We claim.

1. A process for the preparation of enantiomerically pure β -D-(-)-dioxolane-nucleosides comprising preparing the dioxolane ring from 1,6-anhydromannose.
2. The process of claim 1 further comprising converting 1,6-anhydromannose to its (2,3)-isopropylidene derivative.
3. The process of claim 2 further comprising benzoylating the 2,3-isopropylidene derivative of 1,6-anhydromannose to (-)-1,6-anhydro-4-O-benzoyl-2,3-isopropylidene- β -D-mannopyranose.
4. The process of claim 3, further comprising oxidizing the (-)-1,6-anhydro-4-O-benzoyl-2,3-isopropylidene- β -D-mannopyranose to (-)-(2R,4R)-4-(2-benzyloxy-1-hydroxyethyl)-2-(hydroxymethyl)-dioxolane.
5. The process of claim 4, further comprising protecting the 2-hydroxy position of the dioxolane with an oxygen protecting group.
6. The process of claim 5, wherein the oxygen protecting group is selected from the group consisting of trimethylsilyl, dimethylhexylsilyl, t-butyl dimethylsilyl, t-butyl diphenylsilyl, trityl, alkyl groups, acyl groups, benzoyl, p-NO₂ benzoyl, toluyl, methylsulfonyl, and p-toluylsulfonyl.
7. The process of claim 5, further comprising removing the benzoyl group from the 2-hydroxyethyl-position to produce (-)-(2R,4R)-2-(protected-O-methyl)-4-(1,2-dihydroxyethyl)-dioxolane.

8. The process of claim 7, further comprising oxidizing the (+)-(2R,4R)-2-(protected-oxymethyl)-4-carboxyldioxolane to form a compound selected from the group consisting of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)dioxolane.

9. The process of claim 8, further comprising converting (2R,4R)-and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane to a product selected from the group consisting of (2R,4R)- and (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane.

10. The process of claim 1, further comprising condensing the dioxolane ring with a heterocyclic base selected from the group consisting of purine and pyrimidine bases.

11. The process of claim 8, further comprising condensing (2R,4R)-or (2R,4S)-4-acetoxy-2-(protected-oxymethyl)-dioxolane with a heterocyclic base selected from the group consisting of purine and pyrimidine bases.

12. The process of claim 10, wherein the heterocyclic base is selected from the group consisting of adenine, hypoxanthine, N⁶-alkylpurines, N⁶-benzylpurine, N⁶-halopurine, guanine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrimidine, and uracil.

13. The process of claim 11, wherein the heterocyclic base is selected from the group consisting of adenine, hypoxanthine, N⁶-alkylpurines, N⁶-benzylpurine, N⁶-halopurine, guanine, thymine, cytosine, 6-azapyrimidine, 2-mercaptopyrimidine, and uracil.

14. The process of claim 1, wherein the dioxolane nucleoside is $(-)-1-[(2B,4B)-2-(hydroxymethyl)-4-dioxolanyl]thymine$.

15. An enantiomerically pure β -D- $(-)$ -dioxolane nucleoside.

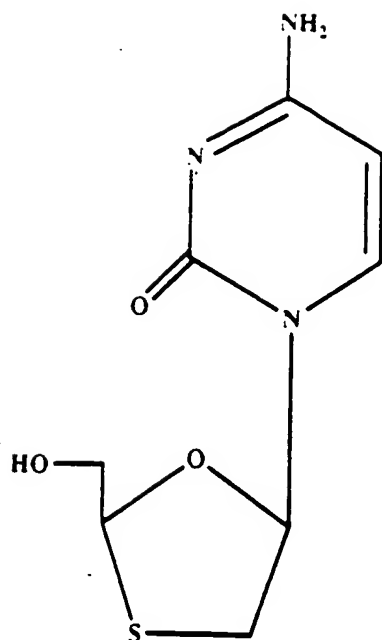
16. The nucleoside of claim 15 that is $(-)-1-[(2B,4B)-2-(hydroxymethyl)-4-dioxolanyl]thymine$.

17. A pharmaceutical composition comprising an amount of an enantiomerically pure β -D- $(-)$ -dioxolane nucleoside that is effective to inhibit HIV in humans.

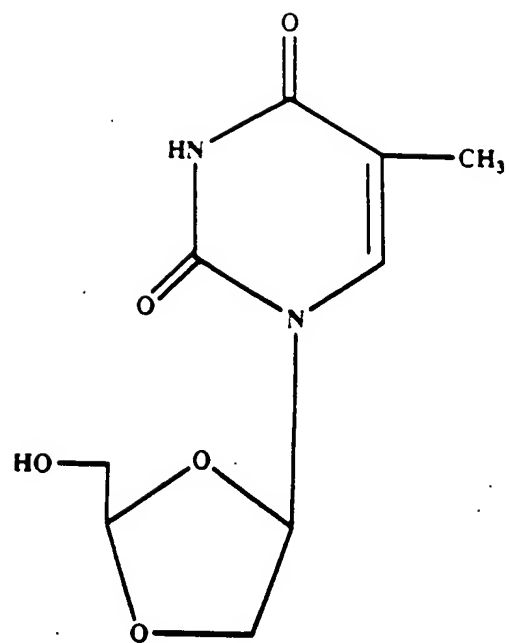
18. The pharmaceutical composition of claim 17, wherein the nucleoside is $(-)-1-[(2B,4B)-2-(hydroxymethyl)-4-dioxolanyl]thymine$.

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(±) -BCH-189



(±) - Dioxolane-T

Figure 1

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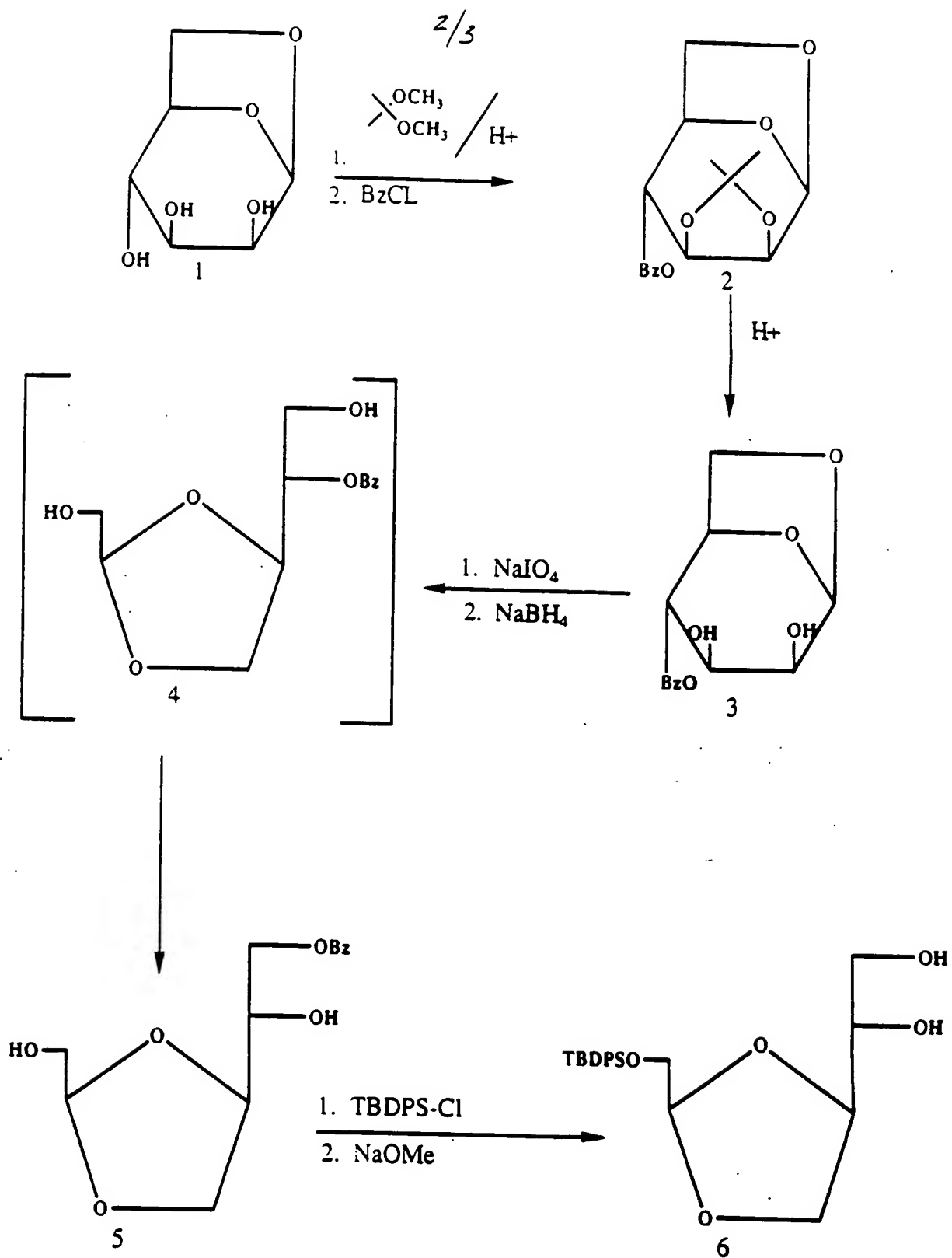
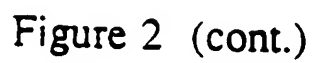


Figure 2

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INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09124

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) *		
According to International Patent Classification (IPC) or to both National Classification and IPC		
IPC(5): C07D 475/00, 487/00, 239/02, 405/04, 473/00; A61K 31/52, 31/505		
U.S. CL: 544/261, 262, 266, 277, 310, 313, 314, 317; 549/214, 358, 397, 449, 453		
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
U. S.	544/261, 262, 266, 277, 310, 313, 314, 317; 549/214, 358 397, 449, 453; 514/265, 266, 274	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
CAS ON LINE		
III. DOCUMENTS CONSIDERED TO BE RELEVANT *		
Category *	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
A, P	US, A, 5,041,449 (IAF Biochem International, Inc.) 20 AUGUST 1991, See entire document.	1-18
A	Chung K. Chu et. al., Journal of Medicinal Chemistry, 1989., Vol. 32, No. 3, pp. 612-617. "Structure-Activity Relationships of pyrimidine nucleosides as antiviral agents for human immunodeficiency virus type 1 in peripheral blood mononuclear cells". See entire document.	1-18
A	Jan Balzarini et. al., Biochemical and Biophysical research communications., Vol. 140, No. 2, 1986, pp. 735-742. "Potent and Selective anti-HTLV-III/LAV activity of 2',3'-dideoxycytidine, The 2',3'- un- saturated derivative of 2',3'-dideoxycytidine" See entire document.	1-18
A	Joseph A. Martin et. al., Journal of Medicinal Chemistry 1990, 33, 2137-2145. "Synthesis and antiviral activity of monofluoro and difluoro analogues of pyrimidine deoxyribonucleosides against human immunodeficiency virus (HIV-1). "See entire document.	1-18
<p>* Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
08 MARCH 1991		20 APR 1992
International Searching Authority		Signature of Authorized Official
ISA/US		For CECILIA TSANG <i>[Signature]</i>

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter ¹² not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out ¹³, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	Phillip A. Furman et. al., Proc. Nat'l., Acad. Sci. USA., Vo. 82, pp. 8333-83337, "Phosphorylation of 3'-azido-3'-deoxythymidine and selective interaction of the 5'-triphosphate with human immunodeficiency virus reverse transcriptase". See entire document.	1-18
A	Patrick Van Roey et. al., J. Am. Chem. Soc. 1988, 110, 2277-2282. "Solid-State Conformation of anti-human immunodeficiency virus type-1 agents: Crystal structures of three 3'-azido-3'-deoxythymidine analogues". See entire document.	1-18
A	Daniel W. Norbeck et. al. Tetrahedron Letters Vol. 30, No. 46, pp. 6263-6266, 1989. "A new 2',3'-dideoxy-nucleoside prototype with in vitro activity against HIV." See entire document.	1-18
P	EP - 382,526 (IAF Biochem international Inc.), 16 August 1990. See entire document.	1-18
A	Roman Z. Sterzychi et. al., Journal of Medicinal Chemistry. 1990, 33, 2150-2157. "Synthesis and anti-HIV activity of several 2'-Fluoro-Containing pyrimidine nucleosides" See entire document.	1-18

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